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Aloe arborescens juice prevents EMF-induced oxidative stress and thus protects from pathophysiology in the male reproductive system *in vitro*

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ABSTRACT

More and more studies suggest that prolonged exposure to EMF may cause adverse biological effects and point directly to a significantly negative correlation between EMF and human health, especially men fertility. In our previous study, we reported that this could be related to the EMF-induced reactive oxygen species formation, followed by DNA damage, cell cycle arrest and apoptosis induction. In this study, we decided to expand our research by the search for substances which would prevent EMF-induced damage in spermatogenic cells. Such an agent seems to be *Aloe arborescens* Mill. juice, which was shown to possess a wide range of protective properties. The administration of *aloe* extract helps among others to prevent the formation of free radicals by various biochemical pathways. Therefore, the main aim of our study was to provide a significant knowledge concerning the mechanism involved in the multi-pathway cytoprotective response of *aloe* juice against EMF. The study was carried out in an *in vitro* mouse spermatogenesis pathway cell lines (GC-1 spg and GC-2 spd). Our results suggest that the *aloe* juice has many positive effects, especially for the cellular antioxidant systems by reducing the intracellular reactive oxygen species pool induced by EMF. In consequence, *aloe* juice prevents DNA damage, cell cycle arrest and therefore the viability and metabolic activity of both cell line tested are preserved. In conclusion, our study provides new insight into the underlying mechanisms through which *aloe* juice prevents spermatogenic cells from cytotoxic and genotoxic events.

1. Introduction

One of the side effects of electronic devices activity is the electromagnetic field (EMF) generation. Increasing exposure to a combination of weak electric and magnetic fields results mainly from human activities and technological progress. The reality is that the level of EMF exposure grows exponentially in the surrounding world. For this reason, all organisms are continuously exposed to different sources of EMF with a wide range of physical parameters (D'Angelo et al., 2015; Dini and Abbro, 2005; Gherardini et al., 2014; Gye and Park, 2012; Lewczuk et al., 2014). Electromagnetic field affects human body as well as any other materials composed of electrically charged particles (Kocaman et al., 2018). Until recent days, the experimental and epidemiological data on the EMF effects have been rare or even contradictory. In particular, the differences relate mainly to the possible molecular mechanisms of cellular response to the EMF, which still remains not fully understood (Consales et al., 2012; D'Angelo et al., 2015; Kocaman et al., 2018; Lewczuk et al., 2014). However, two probabilities have been proposed. The first described as “thermal effect” may result from the tissue temperature increase followed by interference with normal function and development of cells. The second is described as “non-thermal effect” and relates to the cell membrane integrity destruction resulting from the electric current transition. The most realistic mechanism of EMF action seems to be a combination of both mentioned effects (Agarwal et al., 2011; Consales et al., 2012). Furthermore, a number of studies have shown a significantly negative correlation between EMF and human health, especially male fertility. It has been suggested that long-term exposure to EMF may result in a negative impact on the total sperm count, motility and morphology (Agarwal et al., 2011; Gherardini et al., 2014). Recent literature data show that one of the first cellular responses to the electromagnetic field is the formation of reactive oxygen species (ROS) and other free radicals. The upregulation of ROS production may be one of the main causes of cell components, such as lipids, cell membranes and nucleic acids, damage (Consales et al., 2012; D'Angelo et al., 2015; Gherardini et al., 2014; Kiray et al., 2013). Moreover, it is well known that ROS overproduction provokes cell cycle profile changes, protein expression modification or enzymatic and non-enzymatic antioxidants mechanisms dysfunction

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what is the direct pathway to genotoxic events. For this reason, regulation of the redox status is crucial for cell viability or proliferation. Similarly, oxidative stress (OS) plays a major role in the male fertility reduction. Moreover, animal studies have confirmed the reduced value of the total sperm count and the increased percentage of apoptotic cells in the group being exposed to EMF. Thus, it can be concluded that the increased risk of EMF-induced oxidative stress in semen is probable (Agarwal et al., 2012, 2011).

About 20% of the worlds plants have been pharmacologically studied and a significant number of newly available antibiotics originates from natural substances commonly found in nature. According to the World Health Organization (WHO), healing plants are the best source for natural medicines (Sahu et al., 2013). The most important species in traditional medicine are *Aloe barbadensis* Mill. (commonly known as *Aloe vera*) and *Aloe arborescens* Mill. (Radha and Laxmipriya, 2015). Species of *aloe* kind have been recognized as a natural and effective therapeutic agent due to their favorable biological properties all over the world (Radha and Laxmipriya, 2015; Tudose et al., 2009). The phytochemical analysis showed that the *Aloe vera* contains more than 200 chemicals compounds. Moreover, 75 of them have been identified as potentially biologically active. Numerous studies demonstrated that the *Aloe vera* pulp contains proteins, lipids, amino acids, vitamins, enzymes, inorganic and small organic compounds, polysaccharides, as well as significant amounts of antioxidants very likely responsible for therapeutic role in health management (Benson et al., 2015; Channa et al., 2014; Radha and Laxmipriya, 2015; Tudose et al., 2009). Inner gel of the *aloe* leaf is mainly responsible for many biological properties, thus most of the research focuses on the bioactivity of the extract from the *aloe* leaves. Studies show that extract is used to treat many viral diseases and enhance the immune system. Besides, it has been characterized as antibacterial, antifungal and anticancer agent and widely used as local anti-aging agent (Benson et al., 2015; Channa et al., 2014; Radha and Laxmipriya, 2015). In addition, the administration of *aloe* extract helps to prevent the formation of free radicals by various biochemical pathways. Antioxidant activity is probably associated with high levels of polysaccharides and flavonoids of *Aloe vera* leaves. Due to the all beneficial properties, *Aloe vera* seems to be very promising as a universal therapeutic agent, thus further studies are needed to explain the mechanism of action of bioactive chemical substances present in the plant (Benson et al., 2015; Hu et al., 2003; Rodrigues et al., 2016).

In summary, the effect of electromagnetic fields has become an important issue in the field of public health in the context of the male fertility reduction. Due to this fact and the lack of literature data, the main aim of our study was to provide a significant knowledge concerning the mechanism involved in the multi-pathway cytoprotective response of *aloe* juice against EMF using mouse spermatogenesis pathway cell lines. The experiment was based on the *in vitro* model of the meiosis. For this reason, we chose two lines of different cell types at different stages: spermatogonia (GC-1 spg), and spermatocytes (GC-2 spd) as an experimental model of the mentioned process. The experimental setup of the present research was designed to explore the *in vitro* response to 50 Hz frequency of EMF, which humans are most often exposed in a natural environment.

2. Materials and methods

2.1. Cell culture conditions

The mouse spermatogenesis pathway cell lines, GC-1 spg (ATCC Number: CRL-2053) and GC-2 spd (ATCC Number: CRL-2196) were obtained from ATCC (American Type Culture Collection, LGC Standards, UK). Cells were grown and maintained in high-glucose DMEM with 1 mM sodium pyruvate (without L-glutamine) (Corning, NY 14831, USA), supplemented with 10% FBS (Biowest, 49340 Nuaillé, France) and antibiotic mix solution (100 U/ml penicillin, 0.1 mg/ml streptomycin, 29.2 mg/ml L-glutamine) (Biowest, 49340 Nuaillé, France). Cells were kept in standard humidified atmosphere, at 37 °C and 5% CO_2 (Eppendorf New Brunswick Galaxy 170 R CO2 incubator, Germany) and passaged every 3 days. For all procedures, cells were seeded at constant density adjusted to the size of the plastic culture dishes ($1.5 \times 10^3/cm^2$), 24 h before *aloe* juice treatment and EMF exposure.

2.2. Preparation of Aloe arborescens Mill. juice

Three years old *Aloe arborescens* Mill. plants were used. The *aloe* leaves were selected from the mature plants and washed with distilled water. The juice was prepared from fresh leaves. Briefly, the parenchymatous covering the leaves were peeled and the gel drained out, then 0.5 g of freshly obtained gel was used in the further preparation. Tissue was homogenized using high-speed benchtop FastPrep 24 MP sample preparation system and lysing matrix D (MP Biomedicals, Santa Ana, CA 92707, USA) in 100% ethanol (Chempur, Poland). The juice of 1% concentration was used for all experiments and stored in tightly sealed dark containers in the freezer at − 20 °C for later use. Vehicle control groups were treated with the same solvent of the ethanol stock solution, which was used for the test groups.

2.3. Electromagnetic field (EMF) radiation conditions

The electromagnetic waves were generated using unit for low-frequency magnetic field therapy (Magneris, Astar Physiotechnology, Poland). Sinusoidal shape of the magnetic field, as well as continuous and pulsed (1 s/1 s) mode of emission in the 50 Hz frequency, were applied. CP-type flat applicator generates the magnetic flux density in the range of 0–10 mT (0–100 Gs) and 2.5 mT (25 Gs) maximum nominal induction in centre of the applicator. Cells were exposed for 2 h and left 48 h for post-exposure recovery unless it was mentioned otherwise. Control cells were sham-exposed under the same conditions with no electromagnetic field. Highly detailed description of the irradiation set-up EMF exposure system were previously described by Solek et al. (Solek et al., 2017).

2.4. MTT assay

MTT proliferation assay was performed according to the standard protocol described by Mosmann with minor modifications (Mosmann, 1983). In detail, cells were seeded at a density of $1.5 \times 10^3/cm^2$ and after 24 h treated with 1% *aloe* juice and exposed to EMF for 2 h. Next, 48 h after treatment, MTT solution (Cayman Chemical, Michigan 48108, USA) was added to the cell culture medium at a final concentration of 5 mg/ml prepared in PBS (Corning, NY 14831, USA) for 4 h. After incubation at 37 °C, cell culture medium was replaced with 150 µl DMSO (Sigma-Aldrich Sp. z o.o. Poznan, Poland) and absorbance was measured at 595 and 620 nm with a microplate reader (Victor X4 PerkinElmer 2030, PerkinElmer 2030 software v. 4.0 for Windows, Waltham, MA 02451, USA). The results were presented as % of untreated control.

2.5. Cell viability

Fluo Cell Double Staining Kit (MoBiTec GmbH, Germany) combining Calcein-AM and Propidium Iodide-PI was utilized for fluorescent staining of live and dead cells, according to Grudzinski et al. and manufacturer's protocol (Grudzinski et al., 2013). Cellular viability was examined by quantification of the proportion of the cells positive for Propidium iodide staining (red fluorescence produced by interaction with the nucleus of dead cells) over the total number of living cells stained with Calcein-AM (green fluorescence produced by hydrolysis inside the cells). Digital images were taken using InCell Analyzer 2000 and InCell Analyzer Workstation software v. 4.6–12218 for Windows (GE Healthcare, Little Chalfont, UK) and results are presented as % of

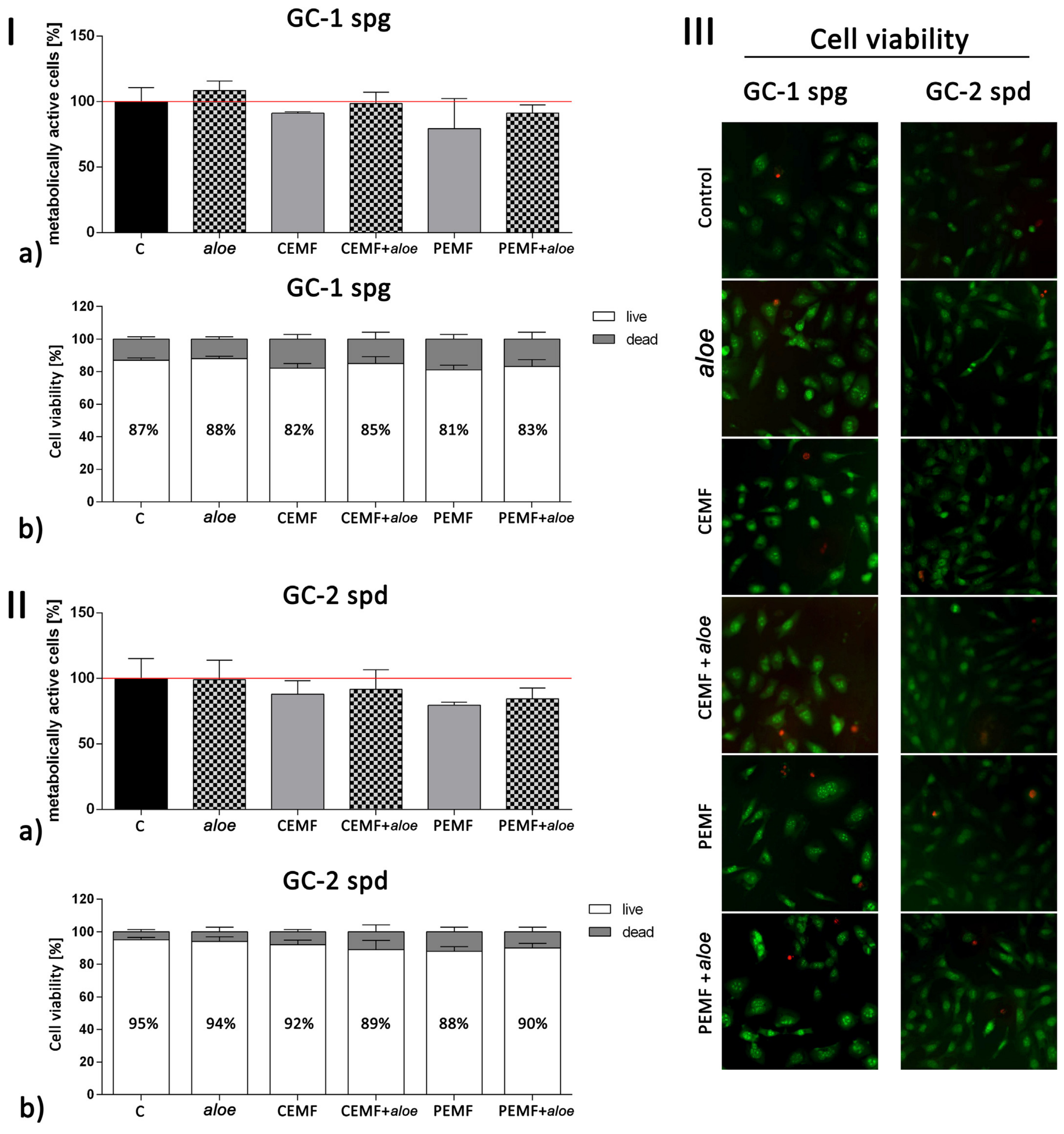

Fig. 1. Display of mitochondrial functions (I-IIa) and cell viability (I-IIb). Representative images of cell viability are also shown (III). Live cells fluoresce bright green, whereas dead cells with compromised membranes fluoresce red-orange. Bars indicate SD, n = 3, no indication – no statistical significance (one-way ANOVA and Dunnett a posteriori test).

total cell number. A minimum of 1000 cells were counted in each sample.

2.6. Analysis of cell cycle position by DNA content and morphological analysis

The present study was designed according to Ferro et al. and Roukos et al. (Ferro et al., 2017; Roukos et al., 2015). We assessed the cell cycle status using DNA Cell cycle plug-in from ImageJ 1.50i software (Wayne Rasband, National Institutes of Health, USA). Briefly, the nuclei were visualized *via* DAPI stain (1 µg/ml) and cell cytoplasm *via* Calcein-AM (0.3 µg/ml). Digital cell images were captured using an imaging cytometer In Cell Analyzer 2000 (GE Healthcare, Little Chalfont, UK). Cell cycle phases in a given cell population were presented as % of cells into G0/G1, S and G2/M phases. A minimum of 1000 cells were counted in each sample. Obtained images were also used for morphological

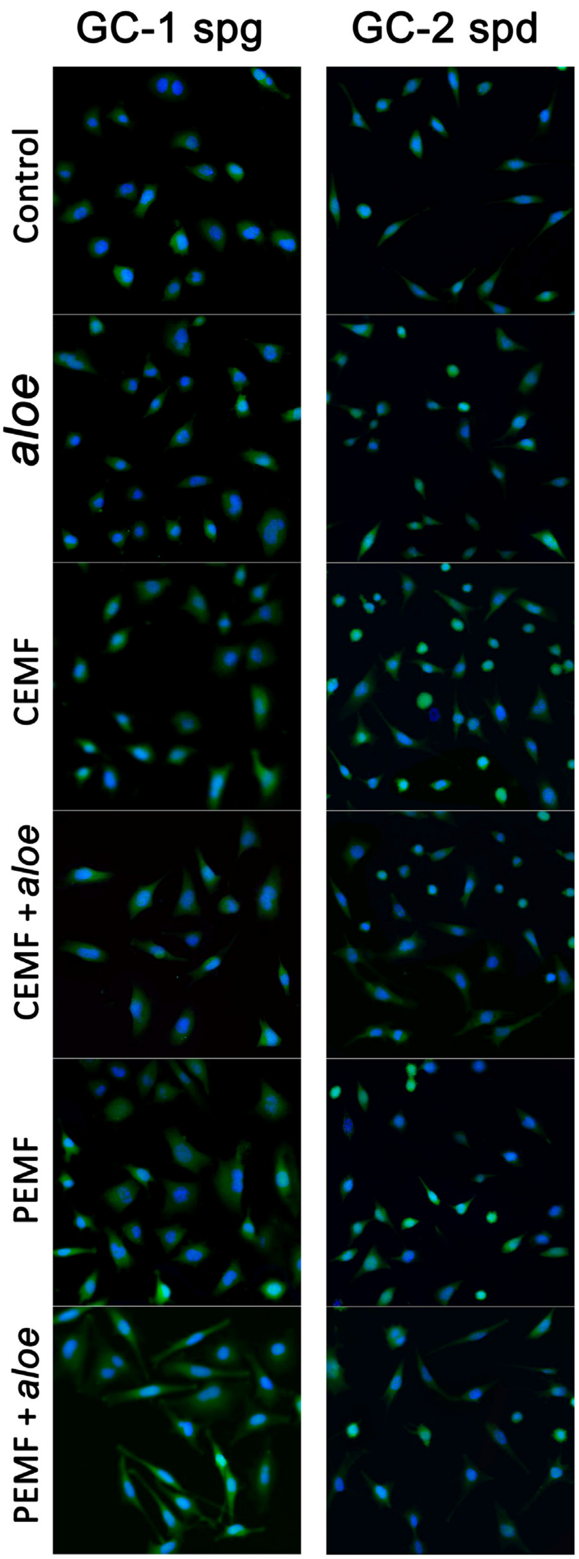

Fig. 2. Alterations in cellular morphology of mouse spermatogenesis pathway cell lines (GC-1 spg and GC-2 spd) treated with *aloe* juice, EMF or combination *aloe* juice + EMF.

changes evaluation.

2.7. Cellular oxidative stress parameters

Measurement of intracellular superoxide anion (O2•-) was evaluated using dihydroethidium probe (DHE; at final concentration 5 μM), while nitric oxide (NO) production was measured using 4-amino-5 methyloamino-2’,7’- difluorofluorescein diacetate probe (DAF-FM; at final concentration 5 μM) according to McCormick et al. and the manufacturer's instructions (McCormick et al., 2016). Superoxide and nitric oxide quantifications were performed using InCell Analyzer 2000 equipped with InCell Analyzer 2000 Software (GE Healthcare, Little Chalfont, UK) and presented as relative fluorescence units (RFU). A minimum of 1000 cells were counted in each sample.

2.8. Western blot analysis

Western blot procedure was previously described by Mytych et al. (Mytych et al., 2017). Briefly, total protein extracts were prepared using ice-cold RIPA buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% NP40, 0.1% SDS, 0.5% sodium deoxycholate and supplemented with 1 mM PMSF just before use. Protein levels of diluted samples were determined using BCA method according to the protocol provided with the manufacturer's instructions (Thermo Fisher Scientific, Rockford, IL 61105, USA). Total protein extract (30 μg) were separated by SDS-PAGE electrophoresis and transferred onto a methanol-activated PVDF membrane (Thermo Fisher Scientific, Rockford, IL 61105, USA). The membranes were blocked at RT in 1% BSA for 1 h, then incubated overnight at 4 °C with one of the primary antibody against: anti-β actin (#PA1–16889), anti-p21 (#PA5–701151), anti-p53 (#700439), anti-NF-κB (#PA5–37658) (Thermo Fisher Scientific, Rockford, IL 61105, USA), anti-Bcl-2 (#sc-7382) (Santa Cruz Biotechnology, Inc. Dallas, Texas 75220, USA), anti-active caspase 3 (#NB100–56113) (Novus Biologicals, LLC, Littleton, CO 80120, USA). Protein of interest was reacted with the appropriate HRP-conjugated secondary antibody: anti-mouse (#A9044) or anti-rabbit (#A0545) (Sigma-Aldrich Sp. z o.o. Poznan, Poland). Immune antigen-antibody complexes were visualized using the ECL western blotting substrate (BioRad, Hercules, California 94547, USA) and Fusion Fx7 system v. 15.15 for Windows (Viber Lourant, Marne La Vallée, France) according to the provided instructions. The relative protein expression levels were normalized to β-actin (GelQuantNET software).

2.9. Statistical analysis

All data were expressed as mean ± SD of three independent experiments. A one-way analysis of variance (ANOVA) with Dunnett post hoc test was used to assess the effects of *aloe* juice and electromagnetic field exposure. The statistical analysis of the results was performed using GraphPad Prism Software ver. 6.01 for Windows. A p-value of < 0.05 was considered as statistically significant between groups.

3. Results

3.1. Effect of EMF and aloe juice on the cellular viability and metabolic activity

The metabolic activity of analyzed cell lines exposed to EMF at a frequency of 50 Hz in both modes of emission was not affected, thus our data suggest that mitochondria preserved their normal function. Furthermore, the *aloe* juice caused a slight increase in metabolic activity in all experimental systems tested, although the results were not statistically significant (Fig. 1. Ia, IIa).

In a subsequent step, we performed a simultaneous fluorescent staining of live and dead cells using the combination of Calcein-AM and Propidium Iodide-PI dyes to determine the changes in viability caused by EMF exposure. As shown in Fig. 1. Ib, IIb continuous electromagnetic field (CEMF) and pulsed electromagnetic field (PEMF) exposure resulted in the decrease in cell metabolic activity and viability while *aloe* juice treatment slightly inhibited the effect of EMF in both cell lines tested in each mode of emission, however, our results were not statistically significant. Moreover, we did not observed changes in total cell count for each experimental model tested relative to the control (Fig. 1. III).

3.2. Effect of EMF and aloe juice on cellular morphology

The morphological characteristics of GC-1 spg cells exposed to PEMF revealed an increase in the cell size with fibroblast-like shape while GC-2 spd cells mostly kept irregularly angular shapes with a scanty cytoplasm. GC-1 spg and GC-2 spd cells treated with CEMF and *aloe* juice maintained morphological features and functional characteristics of established cell lines (Fig. 2.).

3.3. EMF-induced oxidative and nitrosative stress imbalance

Literature data claim that one of the first cellular response to the electromagnetic field is the formation of reactive oxygen species and other free radicals. Based on these facts we were interested in the analysis of oxygen and nitrogen molecular entities modulation to EMF and *aloe* juice treatment. Our results showed significantly higher level of ROS in the cells exposed to a continuous and pulsed EMF compared to control cells $**p < 0.01$ (GC-1 spg cell line) (Fig. 3. Ia). We also noted a relatively low level of ROS (reactive oxygen species) in GC-2 spd cells exposed to CEMF and PEMF as well as treated with 1% *aloe* juice (Fig. 3. Ib). Moreover, in most systems tested, 1% *aloe* juice incubation resulted in the ROS pool reduction. In the case of PEMF, 1% *aloe* juice did not affect ROS levels. However, we noted a significant decrease in the RNS (reactive nitrogen species) in GC-2 spd cells exposed to PEMF $***p < 0.001$. Furthermore, *aloe* juice caused a reduction in the level of RNS compared to cells exposed to PEMF $***p < 0.001$ (Fig. 3. Id, II).

3.4. Cell cycle distribution analysis

Next, we evaluated the changes in the cell cycle phases distribution. Detailed analysis revealed a similar trend for both cell line exposed to EMF. Firstly, we observed a significant decrease in the total number of cells in G0/G1 phase in both modes of emission manner. Moreover, the CEMF caused an S phase elongation, while the PEMF induced G2/M phase elongation. *Aloe* juice treatment resulted in the G0/G1 phase extension compared to EMF treated cells (Fig. 4).

3.5. Protein expression

To reveal molecular mechanisms associated with EMF-mediated actions, next, we analyzed expression of downstream mediators of cellular and biochemical pathways associated with mitochondrial metabolism, viability, oxidative stress balance and cell cycle progression. Figs. 5 and 6 show the expression of caspase 3, Bcl-2, p21, p53 and NF-κB proteins, after exposure to EMF and *aloe* juice in the two-cell line tested. Western blot analysis revealed that level of the caspase-3 and p21 proteins increased in GC-1 spg cells in both modes of EMF emission, while in GC-2 spd cell line only after treatment with pulsed EMF. Furthermore, Bcl-2 level increased in continuous EMF in both cell line tested. The addition of *aloe* juice to cell culture medium resulted in a decrease of caspase-3 activation and simultaneously increased in Bcl-2 level in GC-1 spg cells. Consecutively, the p21 and p53 levels increased after *aloe* treatment in both cell line tested. However, there was no significant correlations between caspase-3, p53 or Bcl-2 protein immunoreactivity and EMF mode of emission. The extent of NF-κB factor activation in GC-1 spg and GC-2 spd cell lines correlates with oxidative stress and NF-κB protein level increased in GC-1 spg cells exposed to CEMF and *aloe* juice. Further, outcomes of the experiments using the methods quantifying ROS and RNS levels were mostly correlated and both of them were consistent with the NF-κB densitometry measurement.

4. Discussion

In this study, we provide for the first time experimental data on the cytoprotective effect of *aloe* juice against EMF action together with a detailed analysis of the molecular mechanisms of intracellular signaling pathways and proteins involved in the response to EMF-mediated stress-related factors.

In detail, we observed a decrease in metabolic activity and viability of CEMF and PEMF-treated cells what is in agreement with others, but we noted that our results were not statistically significant. Furthermore, PEMF was characterized by greater strength of action and resulted in more changes in both cell line tested, which is also supported by observations made by others (Crocetti et al., 2013). Continuing, in control cells in response to treatment with *aloe* juice together with EMF, we observed increased metabolic activity and viability when compared to CEMF and PEMF only. Observed in this study, improvement of normal cells metabolic activity treated with *aloe* juice was suggested also by others (Rahman et al., 2017). The reduction of the cancer cell viability by *aloe* treatment was also shown (Chihara et al., 2015; Guo et al., 2007; Ismail et al., 2013; Jose et al., 2014; Lin et al., 2006; López et al., 2017). The results of other studies on cell viability are confusing. However, the authors agree that the observed differences in cell viability depend on the cell type tested and above all, the EMF parameters of the experimental model. The molecular mechanism remains unclear. The most probable explanation of our results obtained in cell viability decrease and metabolic activity reduction may be the activation of apoptotic cell death in p53 and caspase 3 dependent manners (Lee et al., 2015; Xing et al., 2016). On the other hand, Xu et al., 2017 suggests a decrease in cell viability as a result of the autophagy pathway activation (Xu et al., 2017). The protective properties of *aloe* were also reported by others (Carvalho et al., 2018). Further, decreased metabolic activity of cells exposed to EMF was accompanied by morphological changes and prevented by *aloe* juice treatment, what is also in agreement with others (Nada et al., 2013). As an example, the histopathological examination of rat liver sections and human non-small lung cancer cells (H460) administrated with *aloe* juice showed no morphological changes (Lee et al., 2010; Nada et al., 2013).

The observed decrease in metabolic activity and viability may be associated with apoptosis. One of the most important factors involved in the apoptotic mechanism are caspases. A key role in the regulation of the caspase-dependent pathway of apoptosis is played by external factors such as oxidative stress. They can contribute to the cell self-destruction or its survival (Redza-Dutordoir and Averill-Bates, 2016). In this study, we observed a significant upregulation of caspase-3 expression in both cell lines exposed to CEMF and PEMF. On the other hand, treatment with 1% *aloe* juice contributed to decreased expression of caspase-3 in both lines. Similar observations were done by (Ding et al., 2004; Liu et al., 2015; Sonmez et al., 2010; Tayefi et al., 2010), while the opposite conclusions were presented by (Chen et al., 2014; Joubert et al., 2007). However, this could be due to the different experimental set-up.

What's more, proapoptotic and antiapoptotic proteins from the Bcl-2 family are also involved in the regulation of the caspase function. An upregulation of Bcl-2 expression was observed in both cell lines tested treated with 1% *aloe* juice. A high level of Bcl-2 may be associated with the activation of cell protection mechanisms. In contrast, *aloe* juice was also shown to downregulate Bcl-2 together with p53 upregulation followed by apoptotic cell death in cancer cells (Shalabi et al., 2015).

One of the possible mechanism of *aloe* juice action is based on oxidative stress prevention. Oxidative stress is caused by the lack of balance between reactive oxygen species and antioxidants and contributes to the formation of disorders and damages throughout the cell. Moreover, male gametes are also characterized by their high susceptibility to oxidative stress, and sperm cells are particularly sensitive to damage caused by free radicals due to their structure. Here, we report increased levels of intracellular ROS in GC-1 spg cells and altered levels

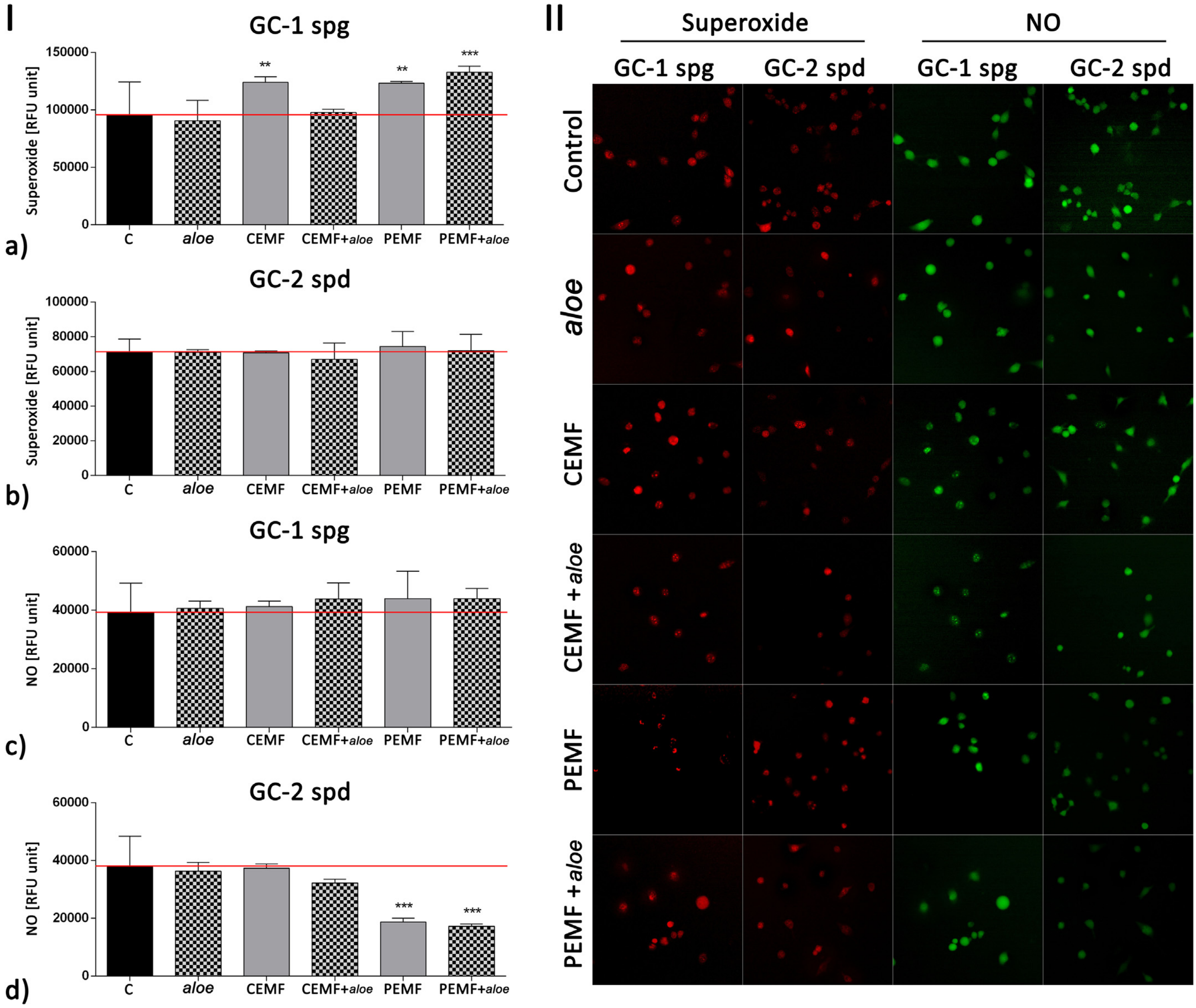

Fig. 3. Correlation between oxidative-nitrosative stress markers (Ia-d). Representative images of superoxide and NO are shown (II). Superoxide-specific forms exhibit red fluorescence while nitric oxide green. Bars indicate SD, n = 3, ***p < 0.001, **p < 0.01, no indication – no statistical significance (one-way ANOVA and Dunnett a posteriori test).

of RNS in GC-2 spd cells treated with CEMF and PEMF, similarly to our previous research (Solek et al., 2017). Undoubtedly, ROS overproduction exerted deleterious effects on the mitochondria function. The EMF-exposure resulted in enhancement of proapoptotic pathways as caspase-3 activation and loss of mitochondrial membrane potential. As a consequence, mitochondria became in turn producer of nitric oxide and superoxide anion (primary reactive oxygen and nitrogen species) thus whole GC-1 spg cell population shifted toward higher intracellular ROS levels even after 48 h of exposure. On the other hand, an explanation of obtained results for the GC-2 spd cell line may be the fact that pulsed exposure causes more cellular damage and the activation of non-enzymatic repair mechanisms which may result in the reduction of total NO pool probably by promoting the activity of NF-κB (Pall, 2016). Interestingly, *aloe* juice reduces the total ROS pools in the most experimental systems, what is in agreement with others (Nada et al., 2013; Wozniak and Paduch, 2012). Furthermore, it was shown that pulsed exposure leads to upregulated ROS production. It seems that in the case of CEMF, the *aloe* juice works. This may be related to the fact that the cells are able to develop a certain adaptive mechanism. However, PEMF in combination with *aloe* juice does exert similar results so it's possible that the ROS overproduction leads directly to oxidative damage in DNA and provokes cell cycle arrest and apoptosis. The obtained results were previously observed by others (Kıvrak et al., 2017; Verdom et al., 2018).

Further, in this study, the cytoprotective action of *aloe* juice associated with oxidative stress prevention is mediated by NF-κB factor. Moreover, the NF-κB upregulation was linked with ROS and RNS decreased production. Next, we confirmed EMF-mediated alterations in the cell cycle profile. We observed the reduction in the population of cells in the G0/G1 phase treated with EMF. In contrast, models with the addition of *aloe* juice were characterized by an increase in the number of cells in the G0/G1 phase and the reduction of the S and G2/M phases, what is in agreement with others (Chihara et al., 2015). Moreover, the outcomes of *aloe* juice treatment were similar to the control. Obtained results may suggest that ROS and RNS overproduction leads to cell cycle arrest, that EMF causes DNA replication and an increase in the number of cells in M phase with a simultaneous constant level of cells in G0/G1 and G2/M phases (Boonstra and Post, 2004; Guo et al., 2014).

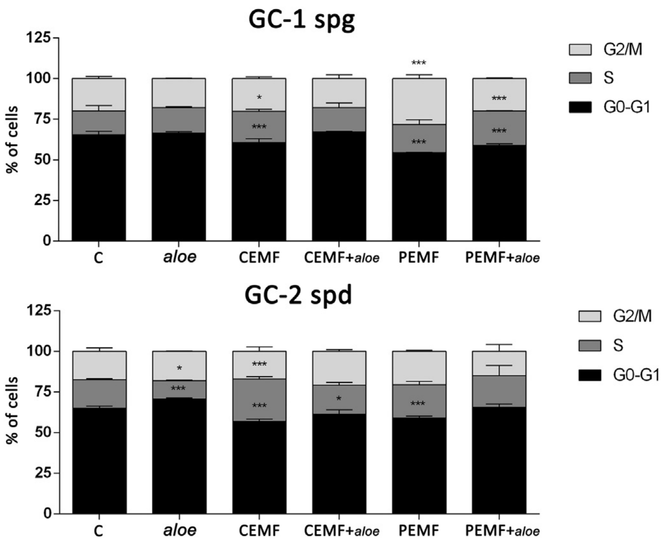

Fig. 4. Alterations in cell cycle profiles of GC-1 spg and GC-2 spd cell lines. Bars indicate SD, n = 3, ***$p < 0.001$, *$p < 0.05$, no indication – no statistical significance (one-way ANOVA and Dunnett a posteriori test).

Aloe-induced G0/G1 preservation is probably linked with upregulated levels of Bcl-2 (Cory et al., 2003). The p53 protein mediates the response to the various types of factors associated with apoptosis, cell cycle arrest, aging, DNA repair and cell metabolism. The high expression level of p53 contributes to the induction of apoptosis with the participation of Bcl-2. Next, the increase in the p53 synthesis may be explained by the cell cycle arrest in order to repair DNA damage, which was reflected in our cell cycle profile analysis. As others previously demonstrated, cell cycle arrest and cell death is associated with the activation of p53 protein and DNA repair (Ashcroft and Vousden, 1999; Harris and Levine, 2005; Levine et al., 2006; Vogelstein et al., 2000). Subsequently, the p21 protein contributes to the arrest of the cell cycle in response to external and internal factors. This protein is also the effector of many anti-proliferative signals. In turn, cell cycle arrest allows DNA damage repair incurred before cell replication. This protein protects against cell death by overexpression of p53 (Abbas and Dutta, 2009). In this study, we performed the analysis of the expression of p21 protein level. The obtained results indicate an increase in p21 expression cells treated with CEMF and *aloe* juice compared to the control. The high level of p21 protein mediates the inhibition of the cell cycle and protection against p53-dependent apoptosis. The ability of *aloe* juice to inhibit DNA damage may be at least in part due to its antioxidative properties (Korkina et al., 2003; Shekhawat et al., 2007).

In summary, based on the presented results, it can be concluded that the *aloe* juice has many positive effects especially for cell antioxidant systems and reduces cellular ROS production. It seems that the main mediator of these changes is oxidative stress. Furthermore, research suggests that direct damage to the genetic material also seems possible (Mihai et al., 2014), however, the formulation of such far-reaching conclusions requires further research.

5. Conclusions

We provide novel evidence that *aloe* juice has many positive effects primarily for cell antioxidant systems. The accumulation of free radicals

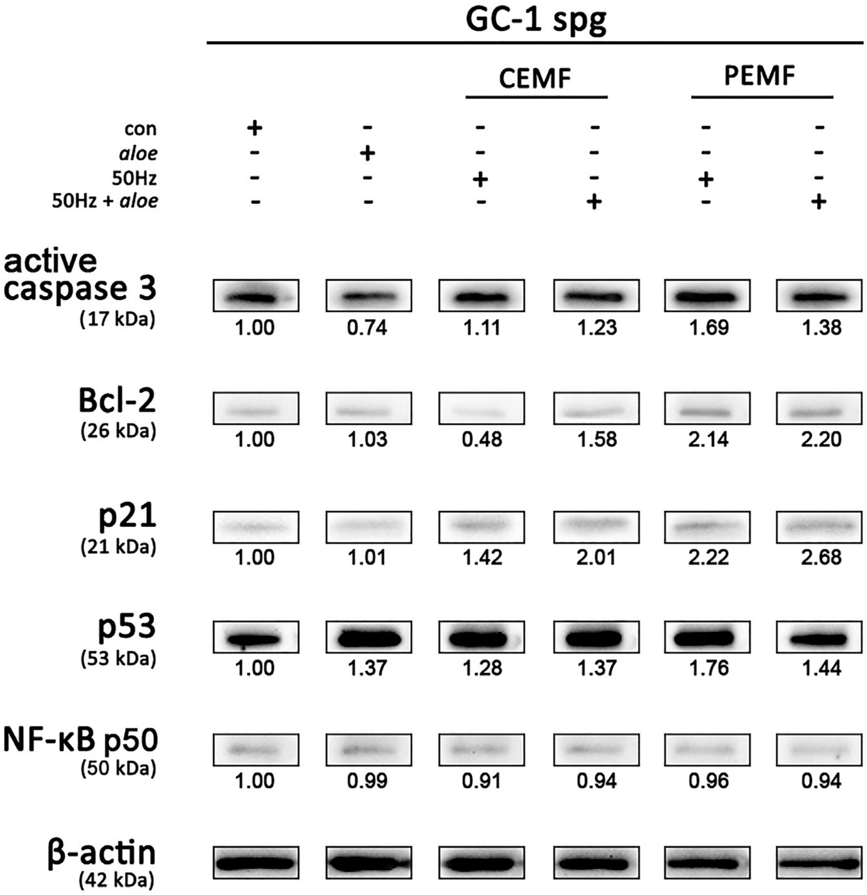

Fig. 5. Representative western blots depicting protein expressions involved in mitochondrial metabolism, viability, cell cycle progression and oxidative stress balance of GC-1 spg cell line. Selected images for each cell pathway are shown. Numbers represent relative density normalized to β-actin.

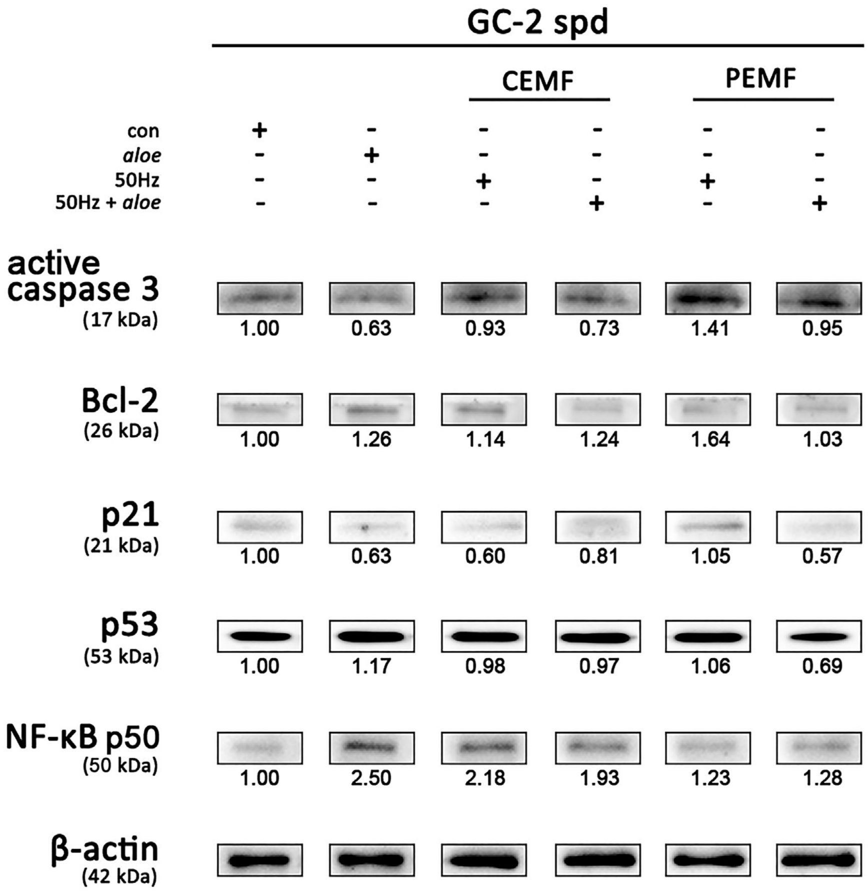

Fig. 6. Representative western blots depicting protein expressions involved in mitochondrial metabolism, viability, cell cycle progression and oxidative stress balance of GC-2 spd cell line. Selected images for each cell pathway are shown. Numbers represent relative density normalized to β-actin.

in testicular tissue and the increase in cellular ROS levels in semen may lead to the reduced male fertility. It is not clear whether EMF has a direct effect on DNA damage through mediator molecules, particularly such as ROS. Our *in vitro* results confirming changes in protein expression do not imply that similar reactions will take place in the human body during exposure to EMF. However, our model may be useful for estimation of the genotoxic and proteomic effects of EMF exposure. To preserve the reproductive potential, it is necessary to develop methods protecting cells from the harmful effects of EMF on living organisms.

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Declarations of interest

None.

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